PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PBA/D088328PWO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 00144	21/01/2000	21/01/1999
Applicant		
ADVANCED MEDICAL SOLUTIONS LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   FIBRES FOR CULTURING EUKARYOTIC CELLS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this int rnational search report, submit comments to this Authority.

6. The figure of the **drawing** to be published with the abstract is Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figur bett r characterizes th inv ntion.

   [X] Non of th figur s.

INTERNATIONAL SEARCH REPORT

[redacted] Application No
PC[redacted]B 00/00144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/00 D01F2/00 //A61L15/00,A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N D01G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 22305 A (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW) 24 August 1995 (1995-08-24) claims ---	1-28
A	WO 97 05238 A (UNIVERSITY COLLEGE LONDON) 13 February 1997 (1997-02-13) claims ---	1-28
A	EP 0 122 793 A (TORAY INDUSTRIES) 24 October 1984 (1984-10-24) claims; figures 1A,2 ---	1-28
A	EP 0 794 223 A (OMIKENSHI COMPANY LTD ET AL.) 10 September 1997 (1997-09-10) -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	19/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Ryckebosch, A

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/00144

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9522305	A	24-08-1995	CA	2182784 A	24-08-1995
			EP	0744932 A	04-12-1996
			JP	10500031 T	06-01-1998
			US	5833641 A	10-11-1998
WO 9705238	A	13-02-1997	AU	716850 B	09-03-2000
			AU	6622996 A	26-02-1997
			CA	2227784 A	13-02-1997
			EP	0842265 A	20-05-1998
			JP	11510690 T	21-09-1999
EP 122793	A	24-10-1984	JP	59192709 A	01-11-1984
			DE	3475084 D	15-12-1988
			US	4639397 A	27-01-1987
EP 794223	A	10-09-1997	JP	2822174 B	11-11-1998
			JP	9241928 A	16-09-1997
			US	5756111 A	26-05-1998

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and Administrative Instructions, Sections 402 and 409)

To:

ATKINSON, Peter, Birch
Marks & Clerk
Sussex House
83-85 Mosley Street
Manchester M2 3LG
ROYAUME-UNI

Date of mailing (day/month/year) 17 July 2000 (17.07.00)	
Applicant's or agent's file reference PBA/D088328PWO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB00/00144	International filing date (day/month/year) 21 January 2000 (21.01.00)
Applicant ADVANCED MEDICAL SOLUTIONS LIMITED et al	

The applicant is hereby **notified** of the following in respect of the priority claim(s) made in the international application.

1. ☐ **Correction of priority claim.** In accordance with the applicant's notice received on: , the following priority claim has been corrected to read as follows:

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

2. ☒ **Addition of priority claim.** In accordance with the applicant's notice received on: 19 May 2000 (19.05.00), the following priority claim has been added:

   GB 21 January 1999 (21.01.99) 9901272.6

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

3. ☐ As a **result of the correction and/or addition** of (a) priority claim(s) under items 1 and/or 2, the (earliest) priority date is:

4. ☐ **Priority claim considered not to have been made.**

   ☒ The applicant failed to respond to the Invitation under Rule 26bis.2(a) (Form PCT/IB/316) within the prescribed time limit.
   ☐ The applicant's notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a).
   ☐ The applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.

   The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim. See Rule 26bis.2(c) and the PCT Applicant's Guide, Volume I, Annex B2(IB).

5. ☐ In case where **multiple priorities** have been claimed, the above item(s) relate to the following priority claim(s):

6. A copy of this notification has been sent to the receiving Office and
   ☒ to the International Searching Authority (where the international search report has not yet been issued).
   ☒ the designated Offices (which have already been notified of the receipt of the record copy).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Eugénia Santos
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/318 (July 1998)

003413161

TENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION RELATING TO PRIORITY CLAIM

(PCT Rules 26bis.1 and 26bis.2 and Administrative Instructions, Sections 402 and 409)

To:

ATKINSON, Peter, Birch
Marks & Clerk
Sussex House
83-85 Mosley Street
Manchester M2 3LG
ROYAUME-UNI

Date of mailing (day/month/year) 17 July 2000 (17.07.00)	
Applicant's or agent's file reference PBA/D088328PWO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB00/00144	International filing date (day/month/year) 21 January 2000 (21.01.00)
Applicant ADVANCED MEDICAL SOLUTIONS LIMITED et al	

The applicant is hereby **notified** of the following in respect of the priority claim(s) made in the international application.

1. ☐ **Correction of priority claim.** In accordance with the applicant's notice received on: , the following priority claim has been corrected to read as follows:

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

2. ☒ **Addition of priority claim.** In accordance with the applicant's notice received on: 19 May 2000 (19.05.00), the following priority claim has been added:

   GB 17 February 1999 (17.02.99) 9903561.0

   ☐ even though the indication of the number of the earlier application is missing.
   ☐ even though the following indication in the priority claim is not the same as the corresponding indication appearing in the priority document:

3. ☐ As a ***result of the correction and/or addition*** of (a) priority claim(s) under items 1 and/or 2, the (earliest) priority date is:

4. ☐ **Priority claim considered not to have been made.**

   ☐ The applicant failed to respond to the Invitation under Rule 26bis.2(a) (Form PCT/IB/316) within the prescribed time limit.
   ☐ The applicant's notice was received after the expiration of the prescribed time limit under Rule 26bis.1(a).
   ☐ The applicant's notice failed to correct the priority claim so as to comply with the requirements of Rule 4.10.

   The applicant may, before the technical preparations for international publication have been completed and subject to the payment of a fee, request the International Bureau to publish, together with the international application, information concerning the priority claim. See Rule 26bis.2(c) and the PCT Applicant's Guide, Volume I, Annex B2(IB).

5. ☐ In case where **multiple priorities** have been claimed, the above item(s) relate to the following priority claim(s):

6. A copy of this notification has been sent to the receiving Office and
   ☒ to the International Searching Authority (where the international search report has not yet been issued).
   ☒ the designated Offices (which have already been notified of the receipt of the record copy).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Eugénia Santos
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/318 (July 1998)

003413162

[redacted]ENT COOPERATION TREAT[redacted]

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 September 2000 (12.09.00)	
International application No. PCT/GB00/00144	**Applicant's or agent's file reference** PBA/D088328PWO
International filing date (day/month/year) 21 January 2000 (21.01.00)	**Priority date** (day/month/year) 21 January 1999 (21.01.99)
Applicant HAMILTON, Douglas, William et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 August 2000 (17.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. *The election* [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia TEFY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 August 2000 (17.08.2000)

PCT

(10) International Publication Number
WO 00/47716 A3

(51) International Patent Classification[7]: C12N 5/00, D01F 2/00 // A61L 15/00, 27/00

(21) International Application Number: PCT/GB00/00144

(22) International Filing Date: 21 January 2000 (21.01.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

9901272.6	21 January 1999 (21.01.1999)	GB
9903561.0	17 February 1999 (17.02.1999)	GB

(71) Applicant *(for all designated States except US)*: **ADVANCED MEDICAL SOLUTIONS LIMITED** [GB/GB]; Road Three, Winsford Industrial Estate, Winsford, Cheshire CW7 3PD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HAMILTON, Douglas, William** [GB/GB]; Heyes Park, Hartford, Northwich, Cheshire CW8 2AJ (GB). **IVES, Christopher, Louis** [GB/GB]; Rose Farm, Stonely Green, Nantwich, Cheshire CW5 8QA (GB). **MIDDLETON, Ian, Philip** [GB/GB]; 7 Kingsley Road, Boughton, Chester CH3 5RR (GB). **ROSETTO, Chiara** [GB/GB]; 20 Vose Close, Hood Manor, Warrington, Cheshire WA5 1EW (GB).

(74) Agent: **ATKINSON, Peter, Birch**; Marks & Clerk, Sussex House, 83-85 Mosley Street, Manchester M2 3LG (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
14 December 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/47716 A3

(54) Title: FIBRES FOR CULTURING EUKARYOTIC CELLS

(57) Abstract: Culturing of eukaryotic cells is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

INTERNATIONAL SEARCH REPORT

Inter[illegible]pplication No
PCT/GB 00/00144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/00 D01F2/00 //A61L15/00,A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N D01G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 97 05238 A (UNIVERSITY COLLEGE LONDON) 13 February 1997 (1997-02-13) claims	1-28
A	EP 0 122 793 A (TORAY INDUSTRIES) 24 October 1984 (1984-10-24) claims; figures 1A,2	1-28
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	19/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

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INTERNAT[redacted] SEARCH REPORT

Information [redacted] patent family members

Inter[redacted] pplication No
PCT/GB [redacted]00/00144

Patent document cited in search report	Publication dat	Patent family member(s)	Publication date
WO 9522305 A	24-08-1995	CA 2182784 A	24-08-1995
		EP 0744932 A	04-12-1996
		JP 10500031 T	06-01-1998
		US 5833641 A	10-11-1998
WO 9705238 A	13-02-1997	AU 716850 B	09-03-2000
		AU 6622996 A	26-02-1997
		CA 2227784 A	13-02-1997
		EP 0842265 A	20-05-1998
		JP 11510690 T	21-09-1999
EP 122793 A	24-10-1984	JP 59192709 A	01-11-1984
		DE 3475084 D	15-12-1988
		US 4639397 A	27-01-1987
EP 794223 A	10-09-1997	JP 2822174 B	11-11-1998
		JP 9241928 A	16-09-1997
		US 5756111 A	26-05-1998

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 5/00, D01F 2/00 // A61L 15/00, 27/00

A2

(11) International Publication Number: WO 00/47716

(43) International Publication Date: 17 August 2000 (17.08.00)

(21) International Application Number: PCT/GB00/00144

(22) International Filing Date: 21 January 2000 (21.01.00)

(30) Priority Data:

9901272.6	21 January 1999 (21.01.99)	GB
9903561.0	17 February 1999 (17.02.99)	GB

(71) Applicant *(for all designated States except US)*: ADVANCED MEDICAL SOLUTIONS LIMITED [GB/GB]; Road Three, Winsford Industrial Estate, Winsford, Cheshire CW7 3PD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HAMILTON, Douglas, William [GB/GB]; Heyes Park, Hartford, Northwich, Cheshire CW8 2AJ (GB). IVES, Christopher, Louis [GB/GB]; Rose Farm, Stonely Green, Nantwich, Cheshire CW5 8QA (GB). MIDDLETON, Ian, Philip [GB/GB]; 7 Kingsley Road, Boughton, Chester CH3 5RR (GB). ROSETTO, Chiara [GB/GB]; 20 Vose Close, Hood Manor, Warrington, Cheshire WA5 1EW (GB).

(74) Agent: ATKINSON, Peter, Birch; Marks & Clerk, Sussex House, 83–85 Mosley Street, Manchester M2 3LG (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: FIBRES

(57) Abstract

Culturing of eukaryotic cells is effected using fibres having at least one open–topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

FIBRES

The present invention relates to fibres and more particularly, but not exclusively, to fibres and materials comprised of such fibres for use in growing or culturing cells, e.g. for the purposes of tissue repair, in-vitro cell cultures and/or organ culture.

Medical devices that are implanted or used on broken skin in wound healing optimally require predictability in their interaction with surrounding tissues and blood. In some cases devices may act as templates for cell in-growth, in some cases they may have already been seeded with cells. Some devices require strong tissue in-growth for fixation, others may function best with minimal interaction. Understanding the biomaterial surface and the cell surface is clearly important in predicting what protein adsorption may occur and how cells may react in terms of adhesion, locomotion or active/passive response/transformation. The key determinants for the biomaterial surface will be surface chemistry in terms of hydrophobic/hydrophilic balance, surface charge, size and direction and counterion, physical size (macroscopic or microscopic), shape (geometry) and surface roughness/smoothness and mechanical properties (elasticity).

Substrates for tissue augmentation or to act as carriers for cultured cell transfer in wound therapy are usually collagen based. In this situation the collagen substrate usually has to be specific to the type of cell growth required and the phenotype and status (secretory, replicatory) grown on the substrate may not turn out to be as required.

It is the specific and non-specific physicochemical forces that will determine gene expression of the cell and phenotype the cell expresses will determine its status capability. It is known for example that cells grown on certain substrates will be inhibited from undergoing terminal differentiation, this has been shown for keratinocytes by Adams & Watt. So the attraction of using non-collagen surfaces for artificial cell substrates is that the cells may retain a migratory and genotypic

capability. The attachment to the cell whatever the substrate, collagen, vitronectin, thrombospondin will be through integrin cell-surface receptor.

Cells seeded onto the surface of a three dimensional structure may only grow in a two dimensional fashion or vice versa depending on the substrate. In some situations, for example nerve regeneration, it is desirable for there also to be not only predictability of attachment and growth but also direction. In the case of nerve regeneration this would mean that an axon could be directed to grow back down its original sheath. Similarly it may be desirable that a cell grow not only in a particular direction, but that a group of cells may grow in the same direction. In the case of voluntary muscle, the muscle belly is made up of muscle cells orientated with their long axes in parallel. Organs such as blood vessels rely on layers of muscle fibres with different layers orientated in a direction at 90° to the previous layer.

Historically work on guiding cells has shown that cells can be directed to migrate along the direction of surface deformations (scratches). Carter et alia (Hapto laxis), can be orientated by fluid shear (Eskin et alia, Ives et alia) by axial strain (Ives et alia). Others have shown that orientation of the Fn molecule can direct cell growth.

US patent 5,610,148 (Robert Brown) entitled "Macroscopically Orientated Cell Adhesion Protein" describes the production of a fibre comprised of fibrils of the cell adhesion protein fibronectin (Fn) that has been denatured and the polymer chains then aligned by unidirectional shear allowing aggregation and precipitation. Cells seeded onto these fibres demonstrate directional cell growth as a result of the longitudinal orientation of the cell adhesion-binding site.

According to a first aspect of the present invention there is provided a method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

The fibres may be used in the form a range of structures for use in providing cell growth, as described more fully below.

In accordance with the first aspect of the invention, therefore, culture of eukaryotic cells is effected on fibres (to the material of which the cells are able to attach) with an open-topped channel formation. Such a formation can be used to provide various advantageous effects for cell culture. Thus, for example, and as described more fully below, the dimensions of the cells may be so related to those of the channel formations that cells locate wholly within the channels and become attached therein to the material of the fibres. Such channel formations are also referred to herein as "troughs". In this case the cells are "protected" from the environment of the bulk phase in which the fibres are provided. Furthermore, the surface of the channel may be treated to provide particular advantageous effects for cell growth. The direction of cell growth may be along the length of the channel. Alternatively, the dimensions of the cells may be so related to those of the channel formations that cells "locate" on the mouth of the channel. Such channel formations are also referred to herein as "grooves". In this case, growth of the cell may be "guided" by the channel.

Routine "in vitro" cell culture has traditionally been carried out on non-porous, flat, 2 dimensional rigid substrates of low surface area to volume ratio. Fibres provide an opportunity to grow cells on high surface area to volume and to improve handling. Conditions of culture and by controlling surface chemistry and geometry of the fibre cell interactions can be predicted.

The cells may, for example, be selected from chondrocytes, cardiomyocytes, osteoblasts, myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchmal origin.

The fibres may be, but are not necessarily, of circular cross-section. The channel may extend generally linearly parallel to the axis of the fibre but other channel configurations which extend along the fibre length are also possible, e.g. helical. The

channel may extend along the full length of the fibre or terminate short of one or both ends thereof. The fibres may have two or more channels extending, for example, generally in parallel to each other. Alternatively the channel may extend transversely to the axis of the fibre.

The fibres may have a length of 1mm to 1000mm (more preferably 5mm-500mm) and preferably a diameter of 10μm-100μm).

It is preferred that the channel has a depth which is not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre but not less than the width of an unspread cell (generally considered to 10μm -20μm. The width of the channel is preferably no greater than half the radius. Such fibres provide a further aspect of the invention.

In certain embodiments of the fibre, the depth of the channel may be less than the width thereof. In other embodiments the channel depth may be greater than or equal to the width of the channel.

Preferred embodiments of fibre in accordance with the invention have a channel whereof the width and depth are each at least 20μm. Such fibres provide a further aspect of the invention.

The channel in the fibres may be of any desired configuration, e.g. U, "rectangular" or "square"-U, or V-shaped. The fibre may, in cross-section, comprise a plurality (e.g. 3) of lobes (preferably symmetrically disposed) and the channel formations are defined between adjacent lobes.

Examples of preferred dimension for a 30μm fibre are shown in the following tables:

Fibre + Groove

Width/Depth	Ideal range of 30μm fibre	Maximum groove size in relation to the fibre dimension	Minimum groove size in relation to cell dimension
Width	5-20μ	1/20 fibre circumference	1/20 cell circumference
Depth	3-10μ	1/10 fibre diameter	N/A

Fibre + Trough

Ideal range of 30μm fibre	Ideal range of 30μm fibre	Maximum trough size in relation to fibre dimension	Minimum trough size in relation to cell dimension
Profile	V.U		
Width	Normally 5-30μ	Fibre diameter <	1 x cell diameter
Depth	Normally 5-30μ	Radius ≤	1 x cell diameter

The above preferred dimensions may be readily adapted for fibre widths other than 30μm.

The fibre preferably comprises a biodegradable biopolymer or a combination of such polymer, examples of which include alginic acid salts (e.g. calcium alginate), carboxymethylcellulose, Methoxypectin, chitosan, chitosan derivatives (e.g. chitosan glutammate), and hyaluronic acid.

The channel of the fibre may have a coating of protein.

Fibres in accordance with the invention may be produced by spinning a solution of the material of which the fibre is to be formed through an orifice into a coagulation bath. The orifice may be configured such that the fibre solidifies into a

form incorporating a channel. Alternatively the orifice may be associated with an engraving device so that the channel is "cut" into the fibre as it is formed. In either case, the coagulation bath may incorporate a material to be adsorbed/absorbed into or onto the fibre surface. Alternative procedures for fibre formation include electrostatic spinning, solvent evaporation, and melt extrusion.

The channel formation ("groove" or "trough") may be formed by the following steps:

(1) The groove or trough is generated by extrusion of the solution through an orifice or a spinneret with a multitude of holes. The orifices may be circular with one or more grooves/troughs or a combination thereof. The grooves have preferably dimension of cell (width 5-10μm) or multiple thereof.

(2) The groove or trough may be produced by engraving the fibre with a sharp blade or engraver. Alternatively the incision can be made by passing the fibre through a rough surface. The roughness is given to the surface by micro-prominences (peaks convexities) having the shape of the groove/trough. They are positioned as the appropriate distance to engrave various fibres. The incision may be made at different stages of the production process of the fibres: after the filament precipitation, after stretching, after the washing, after the drying. It is advisable to groove the fibre after the filament has precipitated out in a short coagulation bath. It will subsequently re-coagulate in its engraved structure in another bath.

(3) The groove/trough can be obtained by etching.

The fibres may be formed into a structure, e.g. random matrices (e.g. non-woven felts and fleeces), orientated matrices (fibres having some relative alignment), knitted structure (e.g. knitted cloths), braided structures (e.g. braided thread), bundled structures, and carded slivers.

In preferred embodiments, microfibrous scaffolds are used that are composed of fibres designed to support cell attachment and directed cell motility and cell growth. The advantage of these fibres is they can be tailored to drive specific cell and tissue performance features by varying the level of surface charge, counterion presence or absence of growth factor, as well as their geometry, fibre orientation and axial strain; various fibres of the embodiments can be envisaged matrices, bundles, tubes, etc. The advantage of matrices made from these fibres is that the fibres can be random or have varying levels of orientation, layers of different types of fibre designed to suit different cell types can also be used. The fibres may also be mixed biodegradable and stable and vary in density and be shaped to form a template such that some mechanical, functional loading could be predicted that as degradation occurred would be counter balanced by appropriate tissue in growth. The advantages of these structures over collagen based substrates is that the seed cells once adhered will secrete and lay down there own extra-cellular matrix. Embodiments of the fibres with cell tracks or cell troughs that ensure that cells positioned at one end of the fibre will deliver to a point along the same fibre may be used in nerve regeneration where the damaged axon needs to grow back to its previous terminus. Besides non-woven matrices, embodiments may be knitted or woven structures that produce sheets or tubes useful in tissue repair or replacement, e.g. dermal substitute, vascular graft. Embodiments utilising the orientational/directional nature of the cell growth on these fibres and fibre scaffolds may be in vitro growth of muscle where muscle cell growth may be unidirectional along parallel fibre.

Following the principles outlined above a range of devices may be constructed from the fibres that are designed to allow cell attachment and growth (although it may be cell type specific) but is unique in that the fibre structure will cause the cell to grow down the fibre and thus be delivered to any point along, or at the other end of the fibre, for which we use the term "guide wire cell growth". The fibre structure that is unique in being able to predict this cell response is one where the bulk fibre maybe made of calcium alginate or other materials as described for different embodiments that has been formed with a groove along its length. The width of the fibre groove would normally be about 1/20 of the unspread circumference of the cell, the size of

the groove would normally be the diameter of an unstuck cell depending on the cell type but large enough to allow the cell to adhere and spread whilst retained within the groove. It is possible that the cell profile may stay entirely within the groove and not be above the circumferential profile of the fibre. In the past various methods have been proposed or actually utilised hollow fibre structures to grow cells and overcome some of the problems of growth on the surface of the fibre alone. The current grooved fibre structure proposed gives the benefits of both a fibre and hollow fibre in that the cells are in direct contact with the media whilst on a fixed and potentially permeable substrate but the level of surface fluid shear or mechanical shear to which the cell is exposed. If cells are placed on the inside of a hollow fibre allowing the cell to feed only by diffusion through the fibre wall there may be problems because of molecular weight cut off and tendency for the lumen of the main fibre to get blocked by cell growth reducing diffusion capability. In this circumstance the indent may be deep enough to be described as a trough. Depending on the size of the groove relative to the cell, the cell will tend to move along it.

The advantages to using an adhesion substrate other than collagen is that the cells once adhered and growing will lay down their own basement membrane and extra cellular matrix proteins, this eliminates the need for collagen specificity and the problems therein. Using synthetic substrates of the right chemistry it is also possible for the status of the cell and its phenotypic versus geotypic expression to be controlled.

By controlling the chemistry of the counterion of the fibre various cell types may preferentially adsorb or be encouraged to move faster or spread/adhere more or tightly to the fibre. Using this fibre structure cells can be guided down into three-dimensional matrices or can be encouraged to arrive at a specific location. Cells of different types can be seeded to grow into different parts of the structure. Cells are thus oriented on the fibre but by orienting the fibre itself all the cells in a structure maybe oriented. Chemotactic/migratory gradients may also be set up along the length of the fibre in order to speed up response.

It has also been shown that cells that are subjected to axial stress will align across the direction of stress so in an embodiment where cells are grown on an elastic fibre and have been allowed to grow into and between fibres to a point where a three cell structure has been formed by applying axial stress to the fibres the cells will be encouraged to align across the fibres to minimise the exposure. Using this technique for instance a structure seeded with muscle fibres could be organised so that the muscle cell contraction will produce a directional contraction and shortening. In some circumstances it may be appropriate to seed cells onto the fibres in a proteinaceous media in another single or multi protein solution depending upon the activity required. Similarly the fibre may be made of a biodegradable material e.g. biopolymers such as alginate or chitosan gelatin guar gum, etc. or synthetic polymers such as polylactic, polyglycolic or a non-degradable such as polyurethane, etc.

Uses of the cell growth procedures in accordance with the invention are set out below.

Wound Therapy

In an embodiment for wound therapy a bundle of fibres with troughs may be seeded with keratinocytes allowed to grow whilst suspended in culture media and then fibres laid singly in parallel across the wound. Cells in the trough are protected from the shear and damage of culture and handling and readily lay down a new basement membrane when placed on the wound.

Bioreactors

In an embodiment for bioreactors, the use of fibres with troughs provides a structure that would provide the ideal mammalian cell bioreactor. Cells can remain below the circumferential profile of the fibre and thus still be exposed to the culture media for diffusion of nutrients but not to the surface shear. For some cell types and with the appropriate substrate it may be possible to use axial stress to stimulate, align disrupt or detach cells depending on the amount of stretch.

Nerve Guidance

In an embodiment for nerve guidance the fibres would be structured with a trough the size appropriate to the axon and bundles of fibres oriented in parallel with the end of the fibre being in the same position at both proximal and distal positions. Fixation of bundle so that the ends were in apposition to the two ends of the cut nerve would allow directional and positional regeneration of the nerve.

Vascular Grafts

Grooved fibres would be formed into a tube by either winding onto a mandrel or knitting. By combining trough fibres with groove fibres cell alignment can be controlled by both fibres direction and axial stress so the desired layers of orientation could be obtained.

The invention is illustrated by the following non-limiting Examples and Figures of the accompanying drawings which show the results of the Examples.

For the Examples, the following procedures were used.

Cell Culture

L929 mouse fibroblast cells for use in an experiment were grown to confluence and then released from the tissue culture dishes by washing with Hepes Saline, followed by treatment with 0.25% trypsin solution. The resulting supernatant in centrifuged and the pellet of cells re-suspended in Dulbecco's modified Eagle's Medium [containing 10% Foetal calf serum, 5% Penicillin/Streptomyocin, 1% ITS (Insulin transferrin selenite)]. If being sub-cultured, then the cells were plated out on tissue culture plates at a 1:5 dilution.

15mg of each fibre type to be tested were weighed out and placed in each well of a 12 well tissue culture dish. In all experiments the fibres were washed in serum

containing media for a period of 24 hours. The experimental controls are cells plated on tissue culture plastic. Cells used for fibre testing were plated out at a density of 80,000 cells per well. All experiments were terminated up to a 72 hour timepoint.

Fixation and Staining

The cells and fibres were washed twice in Phosphate Buffered Saline (PBS) and then fixed using formalin solution (10% neutral buffered) for 10 minutes. The fixative is removed and the cells and fibres washed twice more in PBS. The cells were then stained with Geimsa for 10 minutes, followed by 3, five minute washes in PBS. The cells are then viewed using a Nikon Diaphot microscope and images captured using a JVC DV1 digital camcorder. The images were then downloaded to an Apple Macintosh Power PC Performa 6400/200 and analysis performed using the public domain program NIH image. For the scanning electron microscopy, fixed samples were dehydrated in 100% ethanol for a period of 2 hours. The samples are then sputter coated using a Denton Vacuum desk 1. The samples are mounted on a stub and viewed using a Hitachi S-510 scanning electron microscope. Images are captured using the JVC camera and analysed on the Macintosh computer using NIH image.

Example 1

Crabyon is a commercially available fibre (Omikenshi Co. Ltd., Osaka Japan) that is produced by co-spinning the polysaccharides chitin and chitosan with cellulose (rayon) by the well known viscose process. A number of grades of Crabyon are available with varying ratios of chitin and chitosan to rayon and with various fibre sizes. One such grade having a low (<50%) chitin/ chitosan content, a mean fibre length of 38mm and a diameter of 8-15μ was observed under a scanning electron microscope and was found to have continuous grooves of 1-2μ width along the length of the fibres (see figure 1a). The cell adhesion properties of Crabyon fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the Crabyon fibres was observed after 1 to 2 hours and many cells were elongated and aligned along the grooved fibres. After 72 hours much higher levels of cell attachment and elongation were observed (see figure 2b).

A measurement of the number of aligned and rounded cells adhering to the Crabyon fibres was made by taking an average of the number of adhered cells from 5 fields of view (the results are presented in figure 2c).

Example 2 (Comparative)

Cotton wool (cellulose) fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 10-20μ and to be smooth, flattened with few outstanding surface topographical features (see figure 2). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the fibres after 2hours was found to be very sparse, even after 48hours only a low degree of cell adhesion was observed with no evidence of cell elongation, alignment and spreading apparent.

Example 3

Chitosan, having a degree of de-acetylation >70% (available from Nigerian Fisheries), was spun as fibres from a solution of 3% w/w in 2% aqueous glacial acetic acid. The solution was ejected from a spineret having 20,000 holes each of 150μ diameter, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 10-20μ and to be smooth, cylindrical with few outstanding surface topographical features (see figure 3a). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cell attachment to the fibres after 2hours was found to be significant and spreading was evident but no elongation or alignment of cells was observed. After 48 hours the number of adherent cells had increased greatly but no evidence of elongation or alignment was apparent (see photograph taken from an optical microscope, figure 3b).

Example 4

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 3) from a 1ml insulin syringe through a needle of 35μ outside diameter into a coagulation bath of sodium hydroxide (5%w/w) and the resulting

fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 40-100μ and to be smooth, cylindrical with few outstanding surface topographical features (see figure 4). The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Similar results to that cited in example 3 were obtained with a number of cells found to adhere to the fibres but no evidence for cell elongation and alignment was apparent.

Example 5

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 3) from a 50ml syringe through a needle of 500μ outside diameter that had been bent at the nozzle tip (with the intention of forming a groove in the fibre) into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope (figure 5a), the fibres were found to have a diameter of 200-300μ and to have a central channel of 100-150μ in width. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. Cells were found to attach to the fibre within the channel (see figure 5b)

Example 6

Chitosan fibres were made in the laboratory on a small scale spinning rig by ejecting a solution of chitosan (as specified in example 3) from a 50ml syringe through a spinneret of 1.2cm diameter having 20 holes each of 70μ diameter, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The fibres were observed under the scanning electron microscope, the fibres were found to have a diameter of 20-50μ and to have grooves across the fibre of 5-10μ in width. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above and cells were found to adhere within the grooves of the fibre (figure 6).

Example 7

Chitosan fibres were made in the laboratory by ejecting a solution of chitosan (as specified in example 4) from a 20ml syringe through a spinneret of 1.2cm diameter having 756 trilobal shaped holes, into a coagulation bath of sodium hydroxide (5%w/w) and the resulting fibres were dried. The dimensions of the spinneret were 3 symmetrical legs each of 70μ in length and 25μ in width (supplied by Enka Technica, Coventry, UK). The fibres were observed under the scanning electron microscope (figure 7a), and were found to have channels with a geometry resembling that of the trilobal spinneret. The cell adhesion properties of the fibres were then assessed using L929 mouse fibroblast cells according to the method outlined above. After two hours a high degree of cell attachment was observed with some cells spreading, although there was no evidence of cell elongation or orientation. After 72 hours there was very high cell attachment to the fibres (figure 7b).

Example 8

The procedure outlined in example 8 was repeated but the fibres were maintained in a hydrated state (in de-ionised water) before the cell adhesion properties were assessed. After two hours a high degree of cell attachment was observed with a great deal of cell spreading evident and some cells were observed to be elongated and orientated along the channel of the fibre. After 72 hours there was high cell attachment to the fibres. A comparison of the number of cells adhering to the fibres described in examples 1, 2, 7 and 8 was carried out after 2 hours and 72 hours, with the fibres of chitosan having a trilobal channeled structure proving more adherent (the results are portrayed in figure 8).

Figure Legends For Examples

Figure 1a
Rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi). The fibres 8 to 15 microns in diameter and are cylindrical. The topography on the surface is longitudinal grooves of width 1 to 2 microns.

Figure 2b
Rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi). The fibres 8 to 15 microns in diameter and are cylindrical. The topography on the surface is longitudinal grooves of width 1 to 2 microns. L929 fibroblasts can be seen to be attached to the fibres, one cells is oriented to the groove long axis and the other cell remains spherical, but well adhered.

Figure 1c
Graph depicting the morphology of cells attached to rayon-chitin-chitosan fibres (Crabyon, ex Omikenshi) after a 72 hour timepoint. Cells show a preference to align to the longitudinal groove on the fibres surface. Data represents the mean of 5 fields of view. Error bars=standard deviation.

Figure 2
Cotton Wool composed of cellulose fibres (ex Asda Superstores). The fibres are flat and have a smooth topography. The diameter of the fibres is between 10 and 20 microns.

Figure 3a
Chitosan fibres (MF2, ex AMS) with a level of deaceytlation greater than 70% from routine commercial production. The fibres are cylindrical and have a smooth topography. The diameter of the fibres is between 10 and 20 microns.

Figure 3b
The adherence of L929 fibroblasts on chitosan fibres (MF2, ex AMS) with a diameter between 10 and 20 microns. The cells are well adhered and spread, but exhibit no alignment to the longitudinal axis of the fibre.

Figure 4
Chitosan fibres with a higher deacetylation of 70%, from laboratory production. The fibre is cylindrical in shape and has few surface topographical features. The diameter of the fibres is between 50 and 100 microns. L929 fibroblasts can be seen attached to the surface of the fibre.

Figure 5a
Chitosan fibres with a higher deacetylation of 70%, from laboratory production. The topography of the fibre is a 100 micron wide channel running longitudinally along the long axis. The diameter of the fibres is between 200 and 300 microns.

Figure 5b
L929 fibroblasts adhesion to 100 micron laboratory production chitosan fibres. Cells can be seen in the channel of the fibre.

Figure 6
Chitosan fibres with a greater deacetylation of 70% from laboratory production. The fibres have grooves running across them. The diameter of the fibres was between 20 and 50 microns. Cells demonstrated a good adherence to the fibres and showed orientation to the grooves running across the fibres.

Figure 7a
Chitosan fibres with a greater deacetylation of 70% from laboratory production. The fibres can be observed to be trilobal in shape. The diameter of the fibres was between 150 and 200 microns and the width of the lobes was 70 microns.

Figure 7b
L929 fibroblast adhesion to trilobal chitosan fibres with a greater deacetylation of 70%. Cells were seen to be well attached to the surface of the fibres.

Figure 8
Graph showing the level of adherence of L929 fibroblasts to chitosan fibres of different geometrics. After 72 hours, the dehydrated trilobal chitosan fibres have the greatest level of cell attachement. Hydrated trilobal chitosan fibres also demonstrate a high level of adherence greater than that of dehydrated round chitosan and Craybon fibres.

CLAIMS

1. A method of culturing eukaryotic cells wherein said culturing is effected using fibres having at least one open-topped channel formation on the mouth of which or within which individual cells adhere and grow under the culturing conditions.

2. A method as claimed in claim 1 wherein the fibres have a length of 5mm-500mm.

3. A method as claimed in claim 1 wherein the fibres have a diameter of 5μm-1000μm.

4. A method as claimed in claim 1 wherein the depth of said channels is at least 1μm but not more than 2/3 the diameter (or maximum cross-sectional dimension) of the fibre.

5. A method as claimed in any one of claims 1 to 4 wherein the width of the channel is no greater than half the radius of the fibre.

6. A method as claimed in any one of claims 1 to 5 wherein the channel formation extends longitudinally along the fibre.

7. A method as claimed in any one of claims 1 to 5 wherein the channel formations extend transversely to the longitudinal axis of the fibre.

8. A method as claimed in any one of claims 1 to 5 wherein channel is of U-"square-U", "rectangular-U" or V-shaped cross-section.

9. A method as claimed in claim 6 wherein, in transverse cross-section, the fibres comprise a plurality of lobes and said channel formation is defined between lobes of the fibre.

10. A method as claimed in any one of claims 1 to 9 wherein the cells locate on the open-mouths of the channel.

11. A method as claimed in claim 10 wherein said channel provides for guided growth of the cell along the channel.

12. A method as claimed in any one of claims 1 to 9 wherein the channels are dimensioned such that cells locate wholly within the channel.

13. A method as claimed in any one of claims 1 to 9 wherein the channels are dimensioned such that cells locate partly within the channel and partly above the profile of the fibre.

14. A method as claimed in any one of claims 1 to 13 wherein the fibres are in the form of a scaffold.

15. A method as claimed is claim 14 wherein the fibres are orientated.

16. A method as claimed in claim 15 wherein the fibres of different composition are layered.

17. A method as claimed in any one of claims 1 to 13 wherein the fibres are aligned as parallel on a permeable flat surface.

18. A method as claimed in any one of claims 1 to 17 wherein said cells are selected from chondrocytes, cardiomyocytes, osteoblasts. myoblasts, epithelial cells, endothelial cells, fibroblasts, or cells of a mesenchymal origin.

19. A fibre with an open-topped channel formation the depth of the channel being no more than 2/3 the diameter of the fibre but at least the width of an unspread cell (normally 10-20μ) and width no greater than ½ the radius.

20. A fibre as claimed in claim 19 that allows for cell adhesion and guided migration and growth.

21. A fibre with an open-topped channel formation in the form of a trough where the trough is at least 20 microns wide and 20 microns deep.

22. A fibre as claimed in claim 21 wherein the trough extends along the length of the fibre.

23. A fibre as claimed in any one of claims 19 to 23 in which various levels and gradients types of growth factor have been entrapped allowing diffusion to the surface to control growth.

24. A microfibrous cell scaffold composition comprising a fibre as claimed in any one of claims 19 to 23 for growing cells to produce functional tissue replacements "in vivo".

25. A scaffold as claimed in claim 24 in which fibres are oriented.

26. A scaffold as claimed in claim 24 in which fibres of different composition are layered.

27. Fibres as claimed in any one of claims 19 to 24 aligned in parallel on a permeable flat surface (i.e. a semipermeable film) and seeded with cells.

28. Fibres as claimed in any one of claims 19 to 24 as cell culture substrates for use in bioreactors also for freezing and thawing cells.

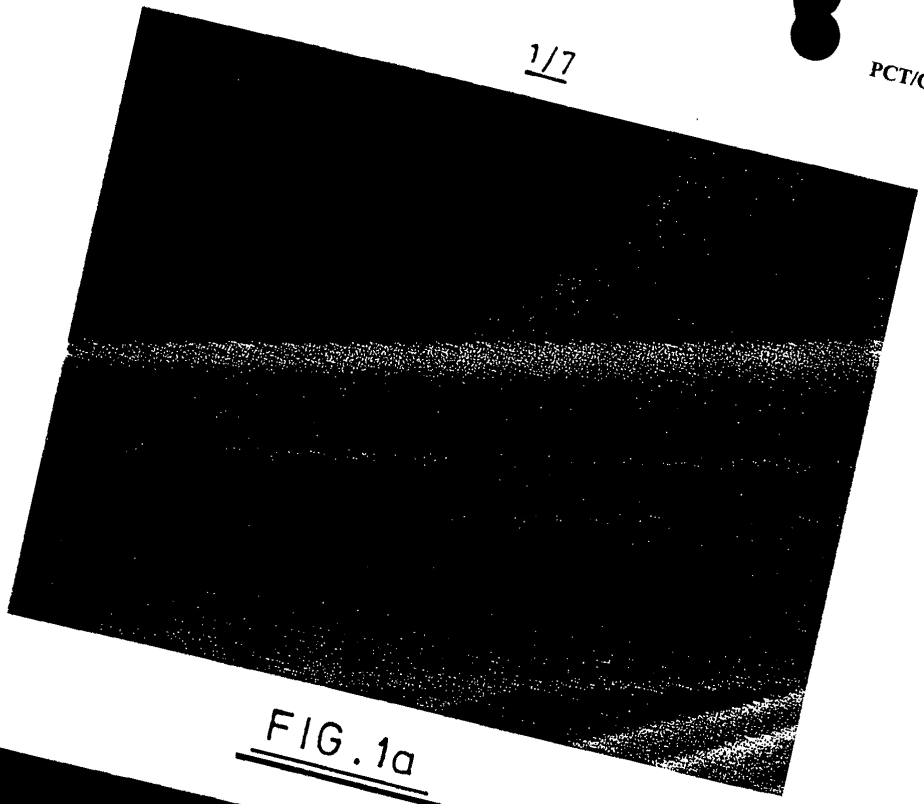


FIG. 1a

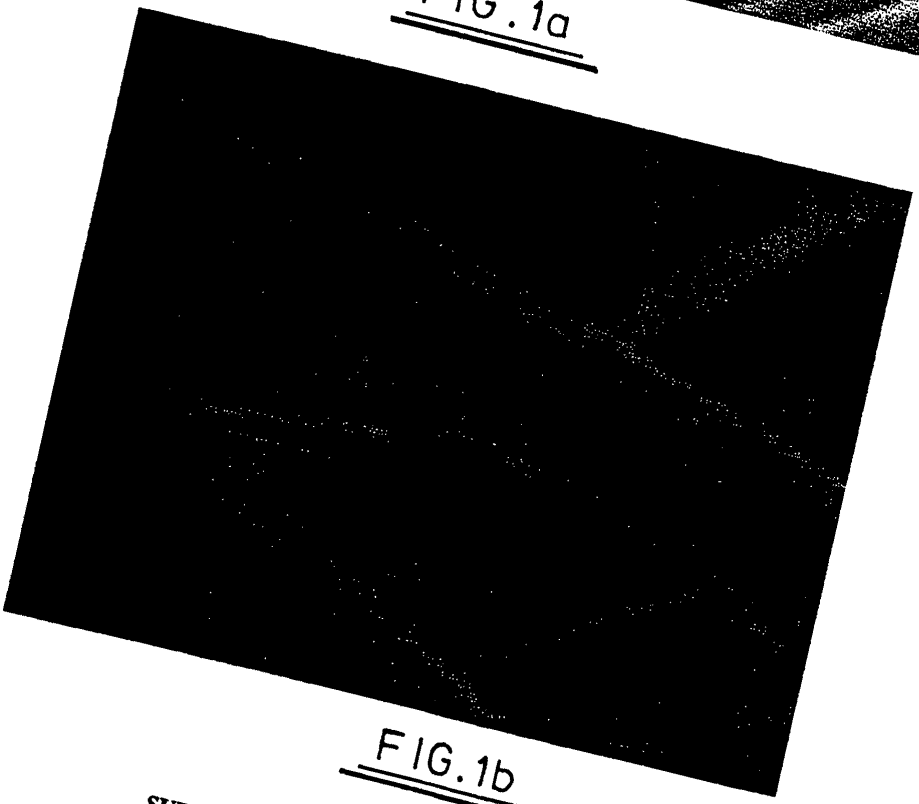


FIG. 1b

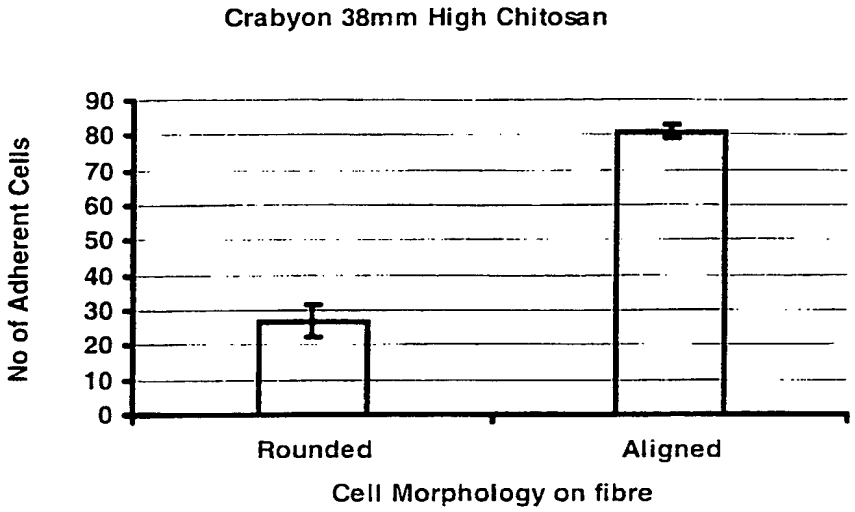

Crabyon 38mm High Chitosan
No of Adherent Cells
90
80
70
60
50
40
30
20
10
0
Rounded
Aligned
Cell Morphology on fibre

FIG. 1c

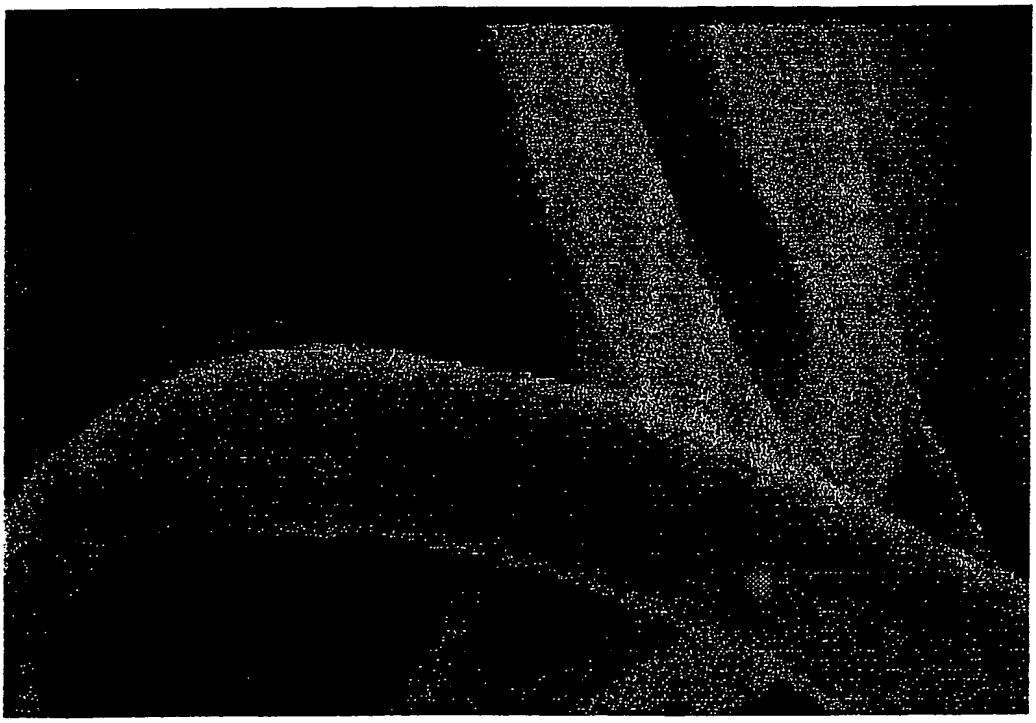


FIG. 2

FIG. 3a

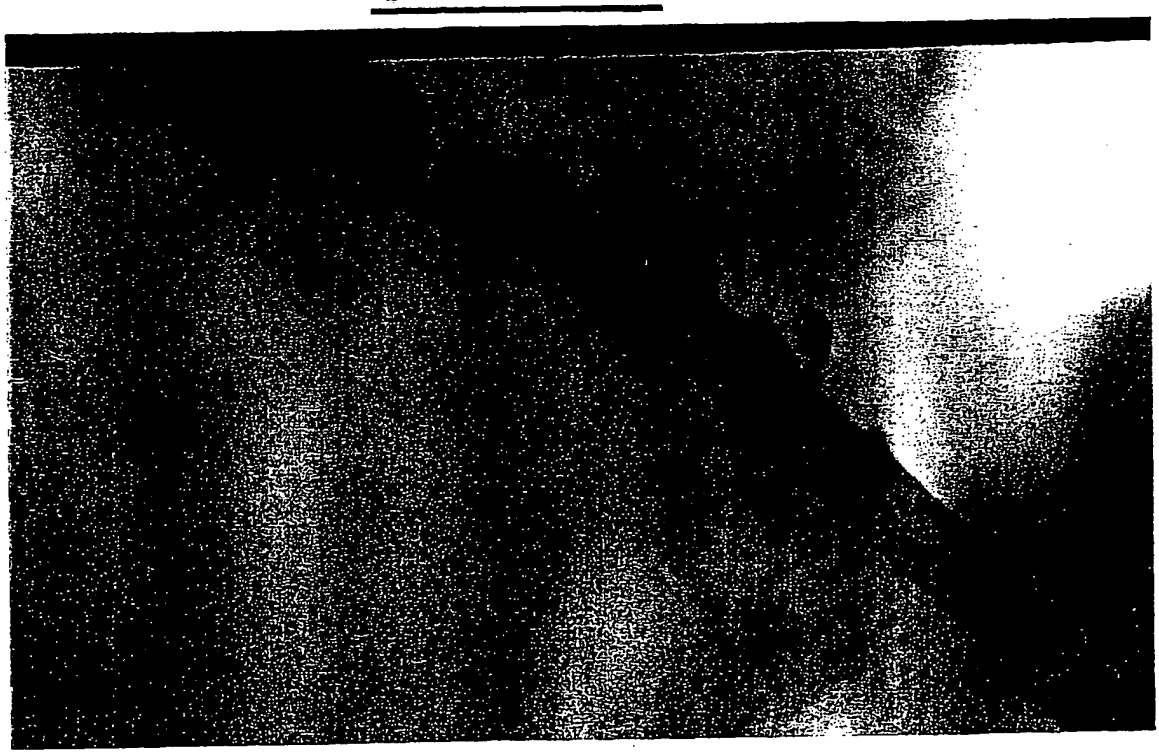


FIG. 3b

FIG. 4

FIG. 5a

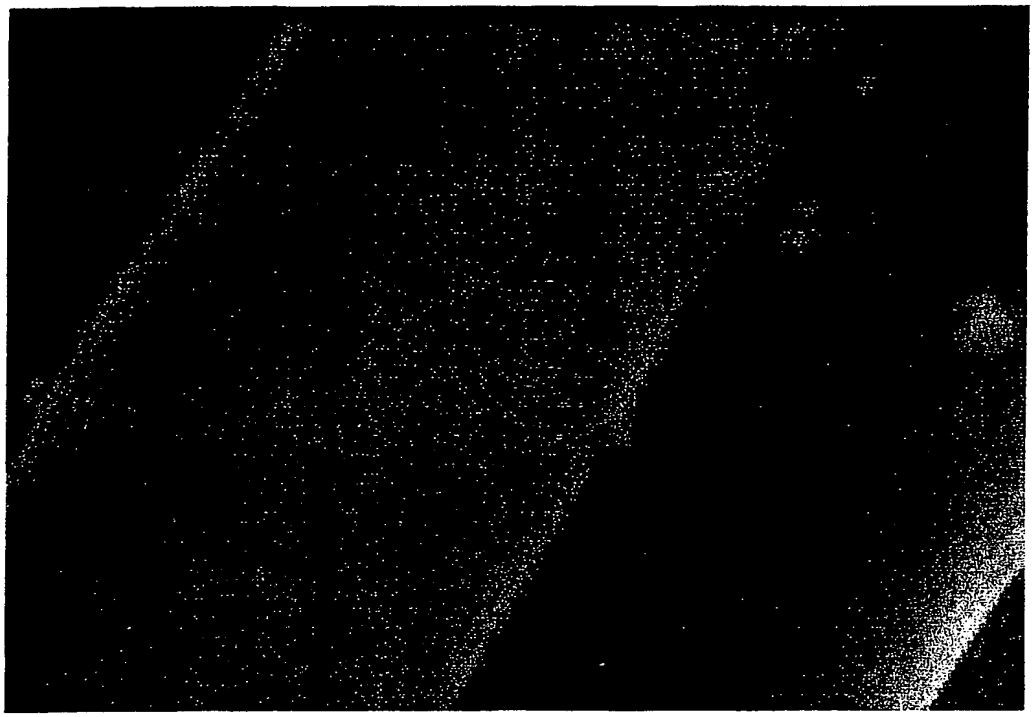


FIG. 5b

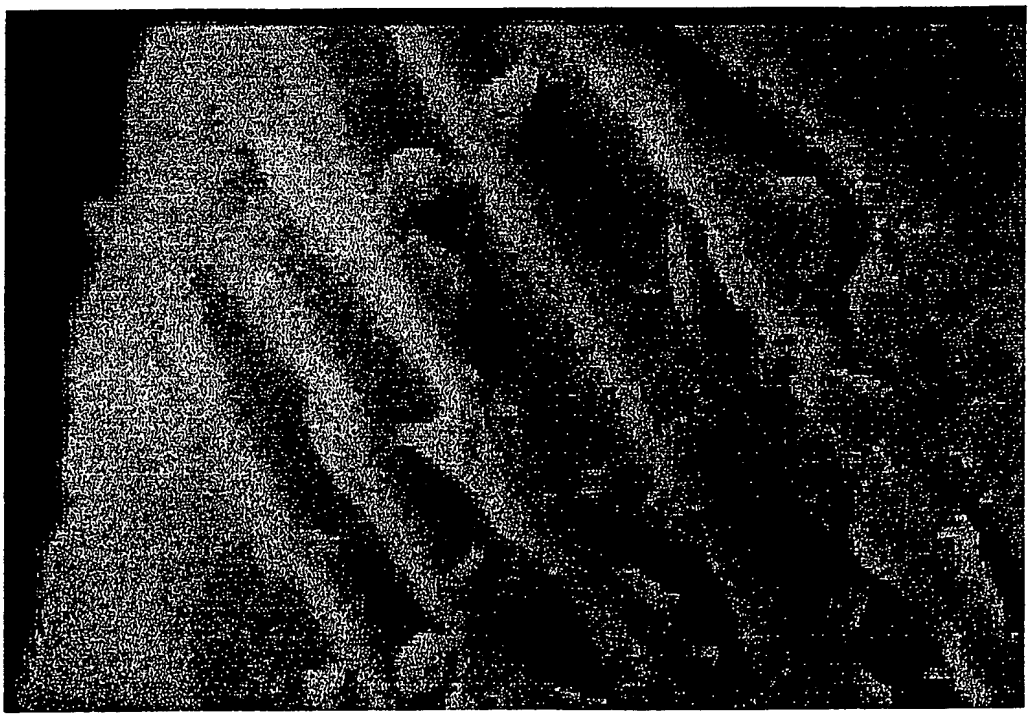


FIG. 6

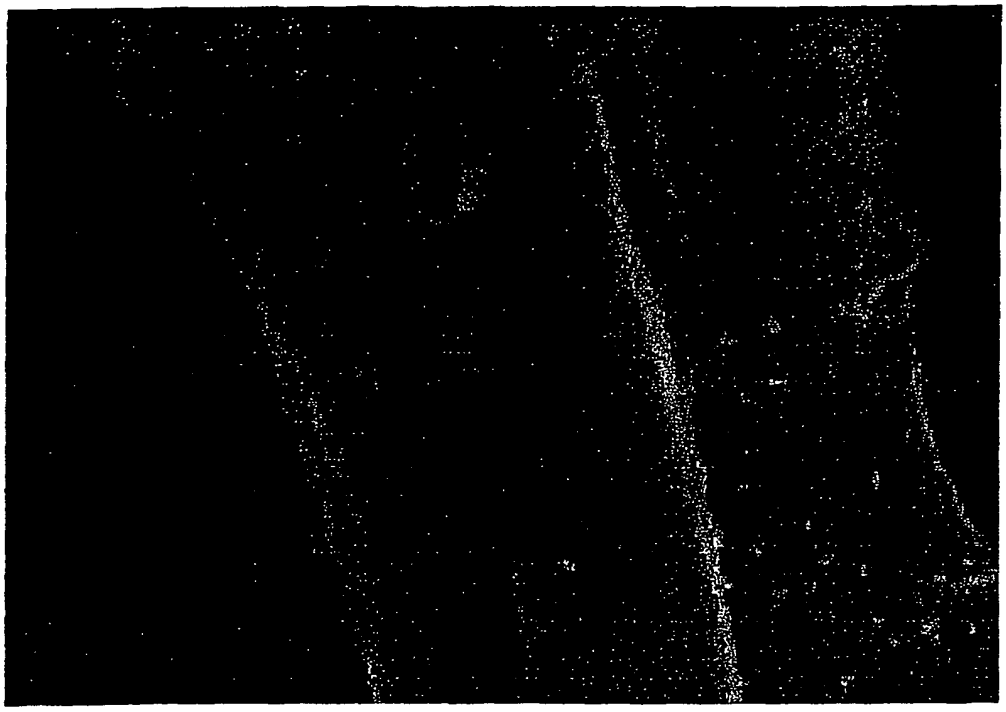
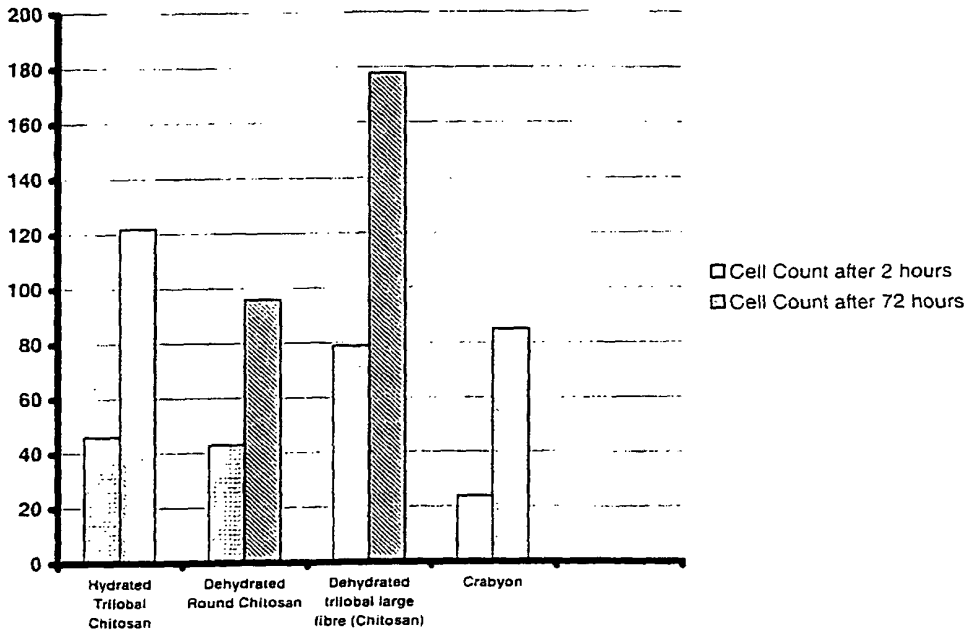


FIG.7a

FIG.7b



Ex. No. 8 3 7 1

FIG. 8

PATENT COOPERATION TREATY

REC'D 15 MAY 2001
WIPO PCT

14

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PBA/D088328PWO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00144	21/01/2000	21/01/1999
International Patent Classification (IPC) or national classification and IPC C12N5/00		
Applicant ADVANCED MEDICAL SOLUTIONS LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/08/2000	10.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Surdej, P Telephone No. +49 89 2399 7334

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00144

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-16 as originally filed

Claims, No.:

1-28 as originally filed

Drawings, sheets:

1/13-13/13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,17,21-23,27
	No:	Claims	1-6,8-16,18-20,24-26,28
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-28

Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO 95 22305 A (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW) 24 August 1995 (1995-08-24)
D2: EP-A-0 122 793 (TORAY INDUSTRIES) 24 October 1984 (1984-10-24)
D3: WO 97 05238 A (UNIVERSITY COLLEGE LONDON) 13 February 1997 (1997-02-13)
D4: EP-A-0 794 223 (OMIKENSHI COMPANY LTD ET AL.) 10 September 1997 (1997-09-10)
D5: RICCI J.L. ET AL.: 'Morphological characteristics of tendons cells cultured on synthetic fibers' JOURNAL BIOMEDICAL MATERIALS RESEARCH, vol. 18, no. 9, November/December 1984, pages 1073-1087

D5 is not cited in the International Search Report but is known to the Applicant.

Introduction

The application discloses a method of culturing eukaryotic cells using fibres having at least one open-topped channel formation, fibres having at least one open-topped channel formation and microfibrous cell scaffold composition comprising said fibres.

Re Item IV
Lack of unity of invention

1. **2** separate groups of inventions are identified:

1. Claims 1-18: A method of culturing eukaryotic cells using fibres having at least one open-topped channel formation on the mouth of which individual cells adhere and grow under the culturing conditions.

2. Claims 19-28: A fibre with an open-topped channel formation the depth of the channel being no more than 2/3 the diameter of the fibre but at least the width of an unspread cell and width no greater than 1/2 the radius and a microfibrous cell scaffold composition comprising said fibre for growing cells to produce functional tissue replacement "in vivo".

2. D1 discloses a method for culturing eukaryotic cells using fibres (e.g. collagen and e.g. page 6, last paragraph to page 7, 3rd paragraph) having at least one open-topped channel formation for cell growth (e.g. abstract, page 3, 5th paragraph, page 9, last paragraph, page 10). D2 discloses fibres with an open-topped channel formation (e.g. abstract, page 5, lines 13-17). Moreover, a fiber (Crabyon) used in example 1 appears to be commercially available (on page 11, last paragraph of the application's description). The subject matter of claims 1 and 19 is not new.

3. The only common technical features which can be distinguished between claims 1 and 19 are **fibres with at least one open-topped channel formation.**

4. Since the said common technical features mentioned in point 1 are known from the prior art documents (see point 2), the subject-matters of claims 1 and 19 are not linked by a common (new and inventive) special technical feature in the sense of Rule 13.2 PCT by taking into account the state of the art.

5. Further detailed analysis of the 2 groups of inventions identified above might reveal an additional lack of unity.

6. In response to the invitation mailed on 16 November 2000, the applicant decided to pay additional fees for the inventions identified by the International Preliminary Examination Authority. Therefore, the International Preliminary Examination Report is established on the entire application as filed.

Re Item V

Reasoned statement under Article 35(2) PCT with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Novelty and inventive step (Art. 33(1)-(3) PCT)

Invention 1: Claims 1-18 (completely).

7. **Claims 1-6, 8-16, 18** are not new in the light of D1, D3 and D5. D1, D3 and D5

disclose a method for culturing eukaryotic cells using fibres having at least one open-topped channel formation (e.g. see point 2 and D1: collagen page 6, last paragraph to page 7, 3rd paragraph, D3: page 3, 2 last paragraphs, page 4, D5: Abstract, materials and methods: synthetic fiber materials, pages 1077, 1084 and 1085). Although, the specific length referred to in claim 2 is not explicitely disclosed in D1, D3 and D5, such dimensions fall implicitly into the scope of said claims for the person skilled in the art. Dimensions and shape as referred to in claims 4-5 and 8-9 of the application are also disclosed in D1 (ex. page 9-10, Fig. 1), D3 (ex. page 3 last paragraph, page 4, 3rd paragraph) and D5 (materials and methods: synthetic fiber materials). From the dimensions of the open-mouths of the channel disclosed in D1, D3 and D5 (see above), the subject-matter referred to in claims 10, 12-13 is not new. The subject-matter of claim 16 is disclosed in D1 (ex. page 12). Cells referred to in claim 18 are disclosed in D1 (ex. pages 16, 19), D3 (ex. page 7, last paragraph) and D5 (ex. page 1074, 3rd paragraph).

8. The subject-matter of **claims 7 and 17** is new but does not involve an inventive step. The features of said claims are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

Invention 2: Claims 19-28 (completely).

9. A fiber as referred to in claim 19 appears to be commercially available (Crabyon) as disclosed in the description of the present application (on page 11, last paragraph) and are also disclosed in D2 (e.g. abstract, pages 2, 5 and 9) and D5 (e.g. abstract, materials and methods: synthetic fiber materials). Therefore, the subject-matter of **claims 19, 20 and 28** is not new.

10. A microfibrous cell scaffold composition comprising a fiber for growing cells to produce functional tissue replacement as referred to in **claims 24-26** is not new in the light of D1 and D3 (ex. D1: pages 7 and 12, D3: page 7, lines 26-28).

11. **Claims 21-23 and 27** are new but are not inventive. The choice of particular dimensions for the trough does not seem to be associated with any particular

effect and consequently represents an arbitrary choice among equally likely alternatives. The fibres of D2, D5 and the commercially available Crabyon fibre are examples of fibres wherein the trough extends along the length of the fibre.

Re Item VIII

Certain observations on the international application

12. The term "fibre" e.g. in claim 1 and 19 which is considered not to meet the requirement of Article 6 PCT. In fact, e.g. any sponge, coral, glass fiber, telephone wire are embraced by the term fibre.

13. The expression "width of an unspread cell" in **claim 19** without being limited by the size introduced by normally lacks clarity under Art. 6 PCT since cells have widely different dimensions and it cannot be seen what dimensions are within the scope of said claim.

14. The orientation of the fibres is not specified in **claims 15 and 25**, consequently the subject-matter of said claims is not limited and for example, randomly oriented fibres fall into the scope of the claim (Art. 6 PCT).

15. From the wording of **claim 19**, it is not clear whether the characteristic "width no greater than the radius" refers to the fibre or the unspread cell. Hence, said claim lacks clarity (Art. 6 PCT).

16. In **claim 28**, the sentence "for use in bioreactors also for freezing and thawing cells" lacks clarity and renders the scope of the claim unclear (Art. 6 PCT).